PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07H 21/00, G01N 33/50, C07K 14/00

A1

(11) International Publication Number: WO 98/38203

(43) International Publication Date: 3 September 1998 (03.09.98)

(21) International Application Number: PCT/US98/02875

(22) International Filing Date: 11 February 1998 (11.02.98)

(30) Priority Data:
60/039,462 27 February 1997 (27.02.97) US

(71) Applicant (*for all designated States except US*): EMORY UNIVERSITY [US/US]; 1380 South Oxford Road, Atlanta, GA 30322 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): GUNN, Robert, B. [US/US]; 2678 Briar Lake Woods Way, Atlanta, GA 30345 (US). TIMMER, Richard, T. [US/US]; 292 Candler Street, Atlanta, GA 30307 (US).

(74) Agents: PRATT, John, S. et al.; Kilpatrick Stockton LLP, Suite 2800, 1100 Peachtree Street, Atlanta, GA 30309–4530 (US).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*With international search report.*

(54) Title: SODIUM–PHOSPHATE COTRANSPORTER IN LITHIUM THERAPY FOR THE TREATMENT OF MENTAL ILLNESS

(57) Abstract

The sodium–phosphate cotransporter existing on virtually every human cell is identified as the same protein as the lithium–sodium

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | | | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mauritania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Malawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Congo | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | NZ | New Zealand | | |
| CM | Cameroon | | | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

# SODIUM-PHOSPHATE COTRANSPORTER IN LITHIUM THERAPY FOR THE TREATMENT OF MENTAL ILLNESS

## BACKGROUND OF THE INVENTION

The subject matter of this present invention was developed in part by one or more grants of the United States Government, NIH HL-28674 and NIH HL-08989.

$Na\text{-}PO_4$ cotransport is the primary mechanism for the regulation of total body phosphate balance. It mediates both the gastrointestinal uptake and renal reabsorption of $PO_4$. Whereas 70% (high phosphate diet) to 85% (low phosphate diet) of dietary phosphate is absorbed by the GI tract the major control is in proximal reabsorption by the kidney of about 70% of the filtered load and in distal discretionary reabsorption of some fraction of the remainder. $Na\text{-}PO_4$ cotransport has been found in the plasma membrane of every mammalian cell examined. In cell membranes it is the principal mechanism for $PO_4$ uptake against the usually negative membrane potential and makes the cytoplasmic and extracellular concentrations approximately equal (±3-fold). Intracellularly, phosphate metabolism includes most important biological molecules: nucleotides, DNA, RNA, glycolytic intermediates, phospholipids, and most proteins through regulatory or structural phosphorylations. Within the mitochondrial membrane $Na\text{-}PO_4$ cotransport is essential for ATP synthesis.

$Na\text{-}PO_4$ cotransporters exist in the kidney, as two isoforms, type I and type II. A related cotransporter is also present in liver where the protein has been partially purified, reconstituted in liposomes, and expressed in oocytes from liver mRNA. Its function in rat hepatocytes in primary culture is stimulated by insulin. $Na\text{-}PO_4$ cotransport activity has been extensively characterized in the duodenum/upper jejunum of many higher vertebrates. Neither the liver nor intestinal forms of the cotransporter

have an isoform of the Type II transporter present in the intestine. Alterations in intestinal $P_i$ reabsorption appear to be related to 1,25-dihydroxy-vitamin $D_3$ status and/or dietary $P_i$ intake. Recently a brain-specific cDNA, designated BNPI, has been cloned that appears to encode a Na-$PO_4$ cotransporter and is 32% identical to the rabbit renal cotransporter, NaPi-1. The brain transporter is specific to the brain and mRNA transcripts are found in the neurons of the cerebral cortex, hippocampus, and cerebellum. The neuronal transporter is also found in peripheral nerves and transports arsenate and $Li^+$ can substitute for $Na^+$. Evidence suggests at least three, possibly four, distinct isoforms of the cotransporter, the renal types I and II, as well as the brain-specific form which may represent a special type. The erythrocyte form represents a third type of Na-$PO_4$ cotransporter, which applicants have discovered is also a retroviral receptor.

Retroviruses require specific cell-surface receptors for cell recognition and infection. Two widely expressed mammalian retrovirus receptors PiT-1 (Glvr-1; Genbank L20859, U.S.Patent 5,414,076) and PiT-2 (Ram-1; Genbank L19931, U.S. Patent 5,550,221) have been cloned and shown to share 30% homology with Pho-$4^+$, a phosphate uptake gene in *Neurospora crassa* and when these two mammalian genes are expressed in oocytes they induce sodium dependent phosphate cotransporter activity. Also, a murine cationic amino acid transporter has been shown to be a retrovirus receptor, thus, indicating there are at least two classes of transporters that are retrovirus receptors. The two sodium phosphate cotransporters/retrovirus receptors (PiT-1 and PiT-2) are widely expressed in tissues and cells (thymus, marrow, lung, liver, heart, kidney, muscle and brain) and appear to be the ubiquitous housekeeping sodium-phosphate cotransporters that every cell requires in order to maintain the intracellular concentration of phosphate above electrochemical equilibrium. Furthermore the cotransporter/receptor isoforms in different species (human and mouse; rat and hamster) together with the differences in retroviral envelope proteins define the species specificity for susceptibility to infection by each

retrovirus. The ability to transfect cell lines from one species with the transporter/receptor isoform from another species and/or alter the envelope protein provides novel model systems and the means to design vectors that allow increased gene transfer into human hematopoietic progenitor cells and other cells.

Two sodium-phosphate cotransporters, PiT-1 and PiT-2, are found in most cells. A third cotransporter BNPI There are different isoforms of these three genes in different people. cDNA and cRNA probes to PiT-1 or to PiT-2 and their mRNA products and antibodies to these proteins distinguish between individuals who are responders or non-responders to lithium treatment.

Applicants have discovered that the sodium-phosphate cotransporter is the same cell membrane protein as the lithium-sodium countertransporter. This discovery has important implications for the diagnosis and therapy of patients in need of lithium for the treatment of manic depression. The present invention provides a readily performed diagnostic test to evaluate patient status, by measuring a combination of sodium, phophate or lithium flux in an in vitro membrane-based translation system.

Applicants have identified the gene product of PiT-1 as the lithium-sodium countertransporter across cell membranes. The PiT-1 gene product is the erythrocyte isoform. Probes for this gene distinguish between responders and non-responders to lithium treatment.

Applicants have also identified the lithium-sodium countertransporter as the physiological mechanism for the extrusion of lithium from cells. It regulates the cell concentration of lithium. The activity of this transporter determines the therapeutic effect of lithium. This invention provides a simple molecular biological test for the ability of cells to extrude lithium. Presently, the only test to determine the activity of a lithium transporter is a laboratory measurement of lithium flux into or out of cells using chemical assays for lithium. See, e.g., Sarkadi, B. et al., *J.Gen.Physiol.*72: 249 (1978).

The lithium-sodium countertransporter has significance for determining the responsiveness of humans with mental disorders to treatment with lithium salts. At

present about half of patients treated with lithium do not improve. There are no techniques at present to diagnose whether a patient will be helped by lithium treatment, except by a time-consuming therapeutic trial. The diagnostic test of the present invention allows genetic screening to predict whether a patient will respond to lithium transport. The test is also a screen for susceptibility to and extent of manic depressive illness. Further, the test is suitable to screen newborns in families with depression for their potential to develop the illness and whether they can respond to lithium treatment.

## BRIEF DESCRIPTION OF THE INVENTION

The sodium-phosphate cotransporter is identified as the same protein as the lithium-sodium countertransporter, and is suitable for diagnostic assays for mental illnesses susceptible to lithium therapy, including manic depression. Various methods for evaluating the flux of lithium and other cations in appropriate cells are also disclosed, including reticulocytes.

## DESCRIPTION OF THE FIGURES

Figure 1 is a schematic diagram of a cell 1 showing that the sodium-phosphate cotransporter is the same cell membrane protein as the sodium-lithium countertransporter.

## DEFINITIONS AND ABBREVIATIONS

$P_i$ Concentration of inorganic phosphate

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a purified DNA molecule coding for a lithium-sodium countertransporter. It also relates to a purified DNA molecule coding for an amino acid sequence selected from the group consisting of hPiT-1, hPiT-2, and hBNPI, said molecule useful for measuring lithium-sodium countertransport in human cells. Specifically, the present invention relates to a novel utility for the sequences identified as SEQ.ID.NO.: 1, SEQ.ID.NO.: 2, SEQ.ID.NO.: 3, SEQ.ID.NO.: 4, SEQ.ID.NO.: 5, and SEQ.ID.NO.: 6.

In one embodiment of the present invention, applicants show that the human amphotrophic retrovirus receptor is useful as a lithium-sodium countertransporter, including the sequences identified as SEQ.ID.NO.: 1, SEQ.ID.NO.: 2, SEQ.ID.NO.: 3, SEQ.ID.NO.: 4, SEQ.ID.NO.: 5, and SEQ.ID.NO.: 6.

In another embodiment of the present invention, there is provided a first method of evaluating sensitivity to lithium therapy in manic depressive patients, comprising the steps of

(a) providing a sample of patient blood;

(b) extracting from the blood sample the patient's DNA;

(c) subjecting the DNA to hybridization with primers specific for any sequence coding for lithium-sodium countertransporter;

(d) polymerizing said sequences, to give polymerized sequences;

(e) amplifying said polymerized sequences, to give an amplified sample of patient sequences;

(f) digesting the amplified sample with one or more restriction endonucleases suitable for mapping sites on the DNA indicating susceptibility to lithium therapy.

In another embodiment of the present invention, there is provided a

(a) providing a sample of patient blood;

(b) extracting from the blood sample the patient's DNA;

(c) subjecting the DNA to hybridization with primers specific for any sequence coding for lithium-sodium countertransporter;

(d) polymerizing said sequences, to give polymerized sequences;

(e) amplifying said polymerized sequences, to give an amplified sample of patient sequences;

(f) subjecting the amplified sample to in vitro membrane-based translation to give a translated sample within a cell; and

(g) subjecting the translated sample to flux analysis of lithium, to evaluate sensitivity to lithium therapy in manic depressive patients.

Specifically, the first and second methods are drawn to sequences to any lithium-sodium countertransporter selected from the group consisting of hPiT-1, hPiT-2, and hBNPI, said sequences identified as SEQ.ID.NO.: 1, SEQ.ID.NO.: 2, SEQ.ID.NO.: 3, SEQ.ID.NO.: 4, SEQ.ID.NO.: 5, and SEQ.ID.NO.: 6.

In another embodiment of the present invention, there is provided a third method of evaluating sensitivity to lithium therapy in manic depressive patients, comprising the steps of

(a) providing a sample of patient blood;

(b) isolating the erythrocytes;

(c) subjecting the erythrocytes to flux analysis of lithium, to evaluate sensitivity to lithium therapy in manic depressive patients.

In another embodiment of the present invention, there is provided a fourth method of evaluating lithium-sodium countertransport in patients with mental illness, comprising the steps of

(a) providing a sample of patient blood;

(b) isolating the erythrocytes;

(c) subjecting the erythrocytes to flux analysis of lithium, to evaluate lithium-sodium.

Phosphorus is a major dietary element essential to most important biological molecules. Its absorption from the gut and reabsorption from the glomerular filtrate is by secondary active transport on a family of $Na\text{-}PO_4$ cotransporters. The gene product responsible for this function in erythrocytes is pharmacologically distinct from the previously characterized renal brush border $Na\text{-}PO_4$ cotransporter. Both the brain and peripheral nerve forms (PiT-1, PiT-2, BNPI) and the red blood cell form of the $Na\text{-}PO_4$ cotransporter can use $Li^+$ as a congener for $Na^+$. Also, arsenate is transported by nerve membranes and probably by red blood cells. Therefore, these two tissues most likely have the same cotransporter/receptor. The renal $Na\text{-}PO_4$ cotransporter, presumably the apical isoform, has been cloned from several species. Applicants have identified the erythrocyte isoform as PiT-1.

Figure 1 schematically shows a simplified diagram, with cell 1. Lithium cation, $Li^+$ enters the cell as the anion $LiCO_3^-$, by the action of the AE1(Anion Exchange Protein, Band 3) countertransporter. Alternatively, the lithium cation leaks into the cells by a minor unknown leak pathway. It is pumped out by the sodium-phosphate cotransporter, which applicants have identified to be also the lithium-sodium countertransporter.

1. Manipulations of DNA for the Preparation of Expression Systems and Other Purposes

Following well known and conventional practice, the hPiT-1 gene or other coding sequences for the lithium-sodium countertransporter are prepared for the expression systems and diagnostic assays of the present invention. These polynucleotide sequences are prepared by ligation of other sequences, restriction endonuclease digestion, cloning, mutagenesis, organic synthesis, or combination thereof, in accordance with the principles and practice of constructing DNA sequences. For sequencing DNA, e.g., verification of a construct at the end of a series of steps, dideoxy DNA sequencing is the preferred method. Other DNA sequencing methods are well known.

Many treatises on recombinant methods have been published, including J.Sambrook et al., Molecular Cloning: A Laboratory Manual 1989; L.G.Davis et al., Basic Methods in Molecular Biology Elsevier 1986; F.M. Ausubel, et al (eds.), Current Protocols in Molecular Biology, Wiley Interscience 1994 (loose-leaf). Such methods include plasmid purification, RNA isolation, Northern blots, Southern blots, Western blots, gel electrophoresis, cDNA library construction, DNA sequencing, amplification by the polymerase chain reaction, cell free translation of mRNAs, and ligation.

Phosphoramidite chemistry in solid phase is the preferred method for the organic synthesis of oligodeoxynucleotides and polydeoxynucleotides. Many other organic synthetic methods are available and are readily adapted to the particular sequences of this invention by a person skilled in the art.

Amplification of DNA or cDNA is a common step in the detection of specific sequences in the diagnostic tests of the present invention. It is typically performed by the polymerase chain reaction (PCR). See, e.g., Mullins, K. et al., U.S. Pat. No. 4,800,159 and other published sources. The basic principle of PCR is the exponential replication of a DNA sequence by successive cycles of primer extension. The extension products of one primer, when hybridized to another primer, becomes a template for the

synthesis of another nucleic acid molecule. The primer template complexes act as substrate for DNA polymerase which, in performing its replication function, extends the primers. The region in common with both primer extensions, upon denaturation, serves as template for a repeated primer extension. The conventional enzyme for PCR applications is the thermostable DNA polymerase isolated from *Thermus aquaticus*, or Taq DNA polymerase. Numerous variations in the PCR protocol exist, and a particular procedure of choice in any given step in the constructions of this invention is readily performed by a skilled artisan. For example, primers for hPiT-1 are organically synthesized, based on its known sequence, and are hybridized to a sample of patient DNA. PCR in combination with reverse transcriptase, so-called RT-PCR, is then carried out to amplify the patient hPiT-1 genes. Subsequent analysis, e.g., by restriction fragment length polymorphism (RFLP), provides information on patient status.

2. Translation of mRNA.

Various techniques have been developed to synthesize or isolate large quantities of capped eukaryotic mRNAs, and are readily adaptable to mRNA coding for hPiT-1 and related sequences. Preferably the source for mRNA is derived from enzymological manipulations, rather than isolation of naturally transcribed mRNA from, e.g., cell lines such as the erythroleukemic cell line K562. Synthetic capped mRNA is preferably prepared by in vitro transcription of the appropriate linearized cDNA constructs containing the appropriate promoter for an RNA polymerase, e.g., T7 RNA polymerase. See, e.g., Fletcher, L. et al., *J.Biol.Chem.*265:19582 (1990), herein incorporated by reference for these purposes. Under these conditions, high yields of capped mRNA coding for hPiT-1, hPiT-2 or BNPI sequences are obtained, which migrate as a discrete band in gel electrophoresis.

The capped mRNA is then subjected to in vitro membrane-based translation, e.g., in *Xenopus* oocytes, microsomes or cultured cells, in an expression system designed to permit flux analysis of Na, $PO_4$, and Li. Preferred expression systems

include *Xenopus* oocytes, and transfected HEK 293 cells. Other suitable transfection systems include *Dictyostelium discoideum* cells, baculovirus-infected ceected Sf9 cells, and CHO cells. Selection of the appropriate cell system, as well as adjusting the experimental parameters to enhance translation, is readily determined within the skill of the art.

3. Construction of expression vector.

The gene for the countertransporter proteins, such as the hPiT-1 gene, is also suitable for expression in an expression vector in a recombinant expression system. Of course, the constructed sequence need not be the same as the original, or its complimentary sequence, but instead may be any sequence determined by the degeneracy of the DNA code. Conservative amino acid substitutions may also be employed, or other modifications, such as an amino terminal methionine.

A ribosome binding site active in the host expression system is ligated to the 5' end of the chimeric coding sequence, giving a synthetic gene. The resulting synthetic gene can be inserted into any one of a large variety of vectors for expression, by ligating to an appropriately linearized plasmid. Expression in *E. coli* is suitable for expression of active lithium-sodium countertransporter protein, e.g., *E. coli* BL21. A regulatable promoter is also suitable for the expression of these coding sequences, e.g., under the control of the *E. coli* lac promoter. Other suitable regulatable promoters include trp, tac, recA, T7, lambda promoters.

4. Diagnostic Assays to Measure Lithium-Sodium countertransport

The flux of a molecule is a measure of the number of molecules that cross the cell membrane per unit time and per unit of membrane (expressed either as area or number of cells or amount of cell protein). The flux is measured by determining the appearance or disappearance (or both) of the molecule on one side of the membrane. The amount on one side of the membrane is measured at different known times either by a chemical determination or by a radioactive determination if a tracer of the atoms

Lithium, atomic number 3, atomic weight 6.9 Daltons, has no radioactive isotopes of use for biological measurements. Chemical detemination must be used instead of a radioisotope. The amount of lithium is most often determined by atomic absorption spectroscopy or emission spectroscopy. The assay of lithium-sodium countertransport flux rate is made by the following steps:

1) a sample of whole blood, e.g. 10 ml, is taken from the patient by venipunture;

2) the cells are mixed in a standard buffered solution containing sodium and lithium chloride solution, and subjected to repeated suspension, centrifugation, removal of supernatant fluid, and resuspension;

3) the cells in the standard solution are incubated in the presence of inhibitors of the Na, K, ATPase (e.g., ouabain at $10^{-5}$ M) and in the presence of inhbitors of Anion Exchange protein (e.g., denitrostilbenedisulfonate at $2.5 \times 10^{-4}$ M), in suspension at body temperature;

4) at given known times samples of cells are removed, cooled on ice to slow the further transport of lithium, then washed 3 times by centrifugation, aspirated to remove supernatant and resuspended in an ice cold lithium-free solution, to give washed cells;

5) the washed cells are lysed with lithium-free water;

6) aliquots of lysed cells are taken and diluted if necessary to measure hemaglobin [(van Kampen, E.J. et al., *Clin.Chim.Acta* 6:538 (1961)] and lithium by flame spectroscopy; and

7) the flux equals the change in lithium per g hemoglobin between samples from the same suspension, divided by the time between samples.

5. Genetic Screening Tests

A variety of methods exist for the evaluation and screening of human DNA sequences obtained as patient samples, for the purpose of patient evaluation. See generally, Caskey, C.T., *Science* 236: 1223 (1987); Bloch, W., *Biochemistry* 30:2735 (1991); Erlich, H.A. et al., *Science* 252: 1643 (1991).

In the classic analysis of polynucleotide sequences by the technique of restriction fragment length polymorphism (RFLP), natural variations in DNA are detected by digestion of DNA, whether or not amplified, with a selected set of restriction endonucleases. The polymorphism need not overlap the site of etiological origin to be evaluated and tested, e.g., the PiT-1 gene, but instead may be a neighboring region linked thereto, e.g., linkage disequilibrium. In one modification of RFLP, a single base pair mutation of a DNA coding strand affects its digestion by a selected restriction endonuclease, and its presence is readily detected by the appropriate primers and PCR (polymerase chain reaction). These types of analytical methods are advantageous because there is no need for a hyribidization reaction of target to labeled probe.

In another technique, known as oligonucleotide complementarity, allele-specific oligonucleotides (ASO) are synthesized for a variety of purposes. These oligonucleotides are useful for either directly hybridizing to target DNA under specific stringency conditions, or for priming in vitro amplification by the polymerase chain reaction.

Tagging or labeling the desired polynucleotide fragments can take various forms. The radioisotope $^{32}P$ and other radioactive labels are not preferred because of laboratory safety and waste disposal requirements. Alternative methods of labeling include chemical analogs, such as biotinylated analogs of TTP and UTP, which incorporate into the resulting DNA and RNA, respectively. The biotin-labeled probe

techniques, or the like. The biotin-labeled probe can also be detected with avidin conjugated to poly AP (calf intestinal alkaline phosphatase), assayed with the appropriate AP substrates. Digoxigenin is a useful substitute for avidin in many applications, and it is readily detected with antibodies specific for digoxigenin. Various combinations of such labels are readily carried out, e.g., a biotin-labeled probe detected with streptavidin conjugated to poly AP, or a biotin labeled probe detected with anti-biotin antibodies linked to AP, or other secondary labeling systems.

6. Preparation of antibodies specific for the lithium-sodium countertransporter protein, and allelic variants thereof.

Monoclonal antibodies are the reagent of choice in the present invention, and a specifcally used to analyze patient cells for specific characteristics of the lithium-sodium countertransporter. Monospecific antibodies to the lithium-sodium countertransporter are purified from mammalian antisera containing antibodies reactive against the lithium-sodium countertransporter or are prepared as monoclonal antibodies reactive with the lithium-sodium countertransporter using the technique of Kohler and Milstein, *Nature* 256: 495-497 (1975). Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogenous binding characteristics for the lithium-sodium countertransporter. Homogenous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope, such as those associated with the lithium-sodium countertransporter, as described above. The lithium-sodium countertransporter specific antibodies are raised by immunizing animals such as mice, rats, guinea pigs, rabbits, goats, horses and the like, with an appropriate concentration of the lithium-sodium countertransporter either with or without an immune adjuvant.

Preimmune serum is collected prior to the first immunization. Each animal receives between about 0.1 mg and about 1000 mg of the lithium-sodium countertransporter associated with an acceptable immune adjuvant. Such acceptable adjuvants include, but are not limited to, Freund's complete, Freund's incomplete,

alum-precipitate, water in oil emulsion containing *Corynebacterium parvum* and tRNA. The initial immunization consists of the lithium-sodium countertransporter in, preferably, Freund's complete adjuvant at multiple sites either subcutaneously (SC), intraperitoneally (IP) or both. Each animal is bled at regular intervals, preferably weekly, to determine antibody titer. The animals may or may not receive booster injections following the initial immunization. Those animals receiving booster injections are generally given an equal amount of the antigen in Freund's incomplete adjuvant by the same route. Booster injections are given at about three week intervals until maximal titers are obtained. At about 7 days after each booster immunization or about weekly after a single immunization, the animals are bled, the serum collected, and aliquots are stored at about -20° C.

Monoclonal antibodies (mAb) reactive with the lithium-sodium countertransporter are prepared by immunizing inbred mice, preferably Balb/c, with the lithium-sodium countertransporter. The mice are immunized by the IP or SC route with about 0.1 mg to about 10 mg, preferably about 1 mg, of the lithium-sodium countertransporter in about 0.5 ml buffer or saline incorporated in an equal volume of an acceptable adjuvant, as discussed above. Freund's complete adjuvant is preferred. The mice receive an initial immunization on day 0 and are rested for about 3 to about 30 weeks. Immunized mice are given one or more booster immunizations of about 0.1 to about 10 mg of the lithium-sodium countertransporter in a buffer solution such as phosphate buffered saline by the intravenous (IV) route. Lymphocytes, from antibody positive mice, preferably splenic lymphocytes, are obtained by removing spleens from immunized mice by standard procedures known in the art. Hybridoma cells are produced by mixing the splenic lymphocytes with an appropriate fusion partner, preferably myeloma cells, under conditions which will allow the formation of stable hybridomas. Fusion partners may include, but are not limited to: mouse myelomas P3/NS1/Ag 4-1; MPC-11; S-194 and Sp 2/0, with Sp 2/0 being preferred. The antibody-producing cells and myeloma cells are fused in polyethylene glycol, about

1000 mol. wt., at concentrations from about 30% to about 50%. Fused hybridoma cells are selected by growth in hypoxanthine, thymidine and aminopterin supplemented Dulbecco's Modified Eagles Medium (DMEM) by procedures known in the art. Supernatant fluids are collected from growth positive wells on about days 14, 18, and 21 and are screened for antibody production by an immunoassay such as solid phase immunoradioassay (SPIRA) using the lithium-sodium countertransporter as the antigen. The culture fluids are also tested in the Ouchterlony precipitation assay to determine the isotype of the mAb. Hybridoma cells from antibody positive wells are cloned by a technique such as the soft agar technique of MacPherson, Soft Agar Techniques, in Tissue Culture Methods and Applications, Kruse and Paterson, Eds., Academic Press, 1973.

Monoclonal antibodies are produced in vivo by injection of pristane primed Balb/c mice, approximately 0.5 ml per mouse, with about $2x10^6$ to about $6x10^6$ hybridoma cells about 4 days after priming. Ascites fluid is collected at approximately 8-12 days after cell transfer and the monoclonal antibodies are purified by techniques known in the art.

In vitro production in anti-lithium-sodium countertransporter mAb is carried out by growing the hydridoma in DMEM containing about 2% fetal calf serum to obtain sufficient quantities of the specific mAb. The mAb are purified by techniques known in the art.

Antibody titers of ascites or hybridoma culture fluids are determined by various serological or immunological assays which include, buy are not limited to, precipitation, passive agglutination, enzyme-linked immunosorbent antibody (ELISA) technique and radioimmunoassay (RIA) techniques. Similar assays are used to detect the presence of the lithium-sodium countertransporter in body fluids or tissue and cell extracts.

It is readily apparent to those skilled in the art that the above described methods

polypeptide fragments of the lithium-sodium countertransporter, or full-length nascent lithium-sodium countertransporter polypeptide, or variants or alleles thereof.

7. Manic-Depression and Other Affective Disorders

The classification of mental illness is fluid and subject to further adjustments and refinements. Two distinct types of mental illness are schizophrenic disorders and affective disorders. Schizophrenic disorders are mental diseases with a tendency toward chronicity and are characterized by psychotic symptoms involving disturbances of thinking, feeling, and behavior. Affective disorders, also known as mood disorders, are psychopathologic states in which a disturbance of mood is either a primary determinant or constitutes the core manifestation. A clinically useful division of affective disorders is bipolar (with periods of depression and elevation) and unipolar (depressions only) mood disturbances. Such bipolar mood disturbances are commonly known as manic depression.

Lithium, usually given as a carbonate salt, attenuates bipolar mood swings, without affecting normal mood. It also appears to be useful in the treatment of aggressive personality disorders, which are typically classified outside of affective disorders. About 50% of bipolar patients respond to lithium therapy. Various clinical attributes are useful in assessing response to lithium, including the presence of manic episodes as the primary mood disorder, an episode frequency of less than about 2 years, as well as past or family history of lithium response. Applicants now provide another attribute to evaluate response to lithium, that is, lithium-sodium countertransport.

8. Lithium Flux Mechanisms

Lithium is commonly used to treat affective disorders. The site of action of lithium is believed to be in the brain. The steady state ratio of intracellular red blood cell lithium concentration to plasma lithium concentration during therapy shows great interindividual variation, although the lithium ratio is relatively constant for any one individual. Individual fluctuations of the lithium ratio have also been reported. The relative constancy of the ratio in an individual may be genetically determined.

The steady state lithium ratio across the red cell membrane is the result of three lithium transport processes: the Na,K,ATPase which is inhibited by ouabain and other cardiac glycosides, the anion exchange protein (AE1, band 3) and the lithium-sodium countertransport system. The Na, K, ATPase pumps Li into the cell by substituting $Li^+$ at a normal $K^+$ binding site, but at therapeutic levels of $Li^+$ (1-2mM) and normal plasma Na and normal plasma K, the Na,K ATPase carries Li poorly (<<0.025 mmol/lit cell • h; <<75 μmol/kg Hgb • h). In plasma like media with 24 mM bicarbonate, the anion exchanger is the principle mediator of the inward leak of Li as the ion pair $LiCO_3^-$. This transport is inhibited by stilbene disulfonates (SITS, DNDS, DIDS), phloretin and dipyridamol. The lithium-sodium countertransporter normally pumps Li out of the cell against its electrochemical gradient so that $[Li]_{cell}$ is lower than $[Li]_{pl}$ and $[Li]_{cell}/[Li]_{pl}$, which lithium ratio is 0.2 to 0.8 in different individuals. A higher steady ratio is expected in alkalosis (higher plasma $HCO_3^-$, $CO_3^{2-}$, and $LiCO_3^-$) due to increased AE1 mediated leak into the cell, or when the lithium-sodium countertransport extrusion of lithium is slowed, either because cell $Na^+$ gradient is decreased or because the countertransporter is less effective.

There is evidence that the differences in the steady state ratio are principally due to differences in the activity of the Na/Li exchanger (lithium sodium countertrnsporter). For example, there is a correlation between the Li influx on the lithium-sodium countertransporter (which is reversible and will run backward given a reversed Na gradient) and the steady state ratio. Also, the steady state Na ratio does not correlate with the steady state Li ratio in different donor cells after 24 hr *in vitro*. Thus the "tightness" of the Na/Li coupling varies among individuals.

Applicants have identified the lithium-sodium countertransporter as the product of PiT-1 gene previously identified as retrovirus receptor and a $NaPO_4$ cotransporter. Applicants have shown that the red cell $NaPO_4$ cotransporter transports Li instead of Na (i.e., $LiPO_4$ cotransport) and that is performs Na/Na exchange and lithium-sodium countertransport.

The lithium ratio has been implicated in the responsiveness of polar disease to lithium treatment, the development of essential hypertension (hypertension of unknown etiology), the susceptibility of individuals to affective (bipolar) disorders, and the toxic side effects of Li therapy.

EXAMPLE 1

KINETIC EVIDENCE THAT THE SODIUM-PHOSPHATE COTRANSPORTER IS THE MAJOR MOLECULAR MECHANISM FOR Na-Li EXCHANGE IN HUMAN RED BLOOD CELLS.

Lithium influxes, $^{32}PO_4$ influxes, sodium effluxes were measured in human red blood cells incubated in an isotonic media containing (mM): 150 (Na + Li + K)Cl, 0.3 $K_2HPO_4$, 20 HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 0.25 DNDS (4,4'-dinitro stilbene-2,2'-disulfonate to inhibit the Anion Exchange Protein Band3 pathway for phosphate transport), titrated to pH 7.64 with KOH at 20°C to give a pH of 7.40 at 37°C where the fluxes were performed. First, external lithium (in the absence of sodium) activated phosphate influx. Lithium activation of phosphate influx was increased by 1, 7.5 and 75 mM external sodium. Second, external lithium stimulated Na efflux in the presence of $10^{-5}$ M ouabain (an inhibitor of the Na-K pump) and was further stimulated by external phosphate. These results indicate that the majority of sodium efflux is on the sodium-phosphate cotransporter. Third, external phosphate concentrations slightly inhibited lithium influxes at low (0.1 -0.3 mM) phosphate concentrations. Fourth, arsenate inhibited sodium-phosphate cotransport in red blood cells with a Ki of 5.2 mM, more than 10 fold greater than in HEK-293 cells, which have a renal ype II sodium-phosphate cotransporter. The collective results indicate that a mechanism for Na-Li exchange on the sodium-phosphate cotransporter.

EXAMPLE 2

KINETIC CHARACTERIZATION OF SODIUM-PHOSPHATE COTRANSPORTER IN THE ERYTHROLEUKEMIC CELL LINE K562: IDENTIFICATION OF THE ERYTHROCYTE SODIUM PHOSPHATE COTRANSPORTER AS hPiT-1.

Na-dependent $^{32}PO_4$ influx into the erythroleukemic line of K562 cells was measured. The $^{32}PO_4$ influx was linear with time of over 30 minutes and was activated over 100-fold by 140 mM Na compared to isomolar substitution by 140 mM N-methyl-D-glucamine. The activation of $^{32}PO_4$ influx by extracellular phosphate (pH 7.4 at 37° C) was hyperbolic with a $K_m^{PO4}$ = 0.36mM and $V_{max}$ = 4500 nmol $PO_4$/g protein - min in 140 mM Na+. The $K_{1/2}^{Na}$ = 40 mM when $PO_{40}$ was 0.3 mM. There was no activation of phosphate influx by Rb, K, or Cs. However, 140 mM Li activated phosphate influx to 18.7% of that realized in the presence of 140 mM Na. The $K_i$ value for arsenate inhibition of Na-dependent $^{32}PO_4$ influx K562 cells was 2.6 mM. These and other kinetic characteristics of sodium-phosphate cotransport in K562 cells are identical to those previously described for human erythrocytes. See Shoemaker et. al., J. Gen. Physiol, 92:449 (1988).

EXAMPLE 3

MOLECULAR IDENTIFICATION OF THE SODIUM-PHOSPHATE COTRANSPORTER IN ERYTHROLEUKEMIC CELL LINE K562 AND ERYTHROCYTES AS HPIT-1.

Human PiT-1 (hPiT-1) was cloned as the human isoform of the gibbon ape retrovirus receptor [Van Zeijl, M., et al (Proc. Nat. Acad. Sci. 91:1168 (1994)]. PCR primers were designed to amplify either the hPiT-1 or hPiT-2 isoforms. The 1700 bp product was amplified by RT-PCR from total RNA isolated from K562 cells, and

Example, indicates that hPiT-1 is the sodium-phosphate cotransporter isoform present in both K562 cells and erythrocytes.

RT-PCR was carried out using 1 μ g of total RNA isolated from K562 cells using the method of Chomczynski et al., Analytical Biochem. 162:156 (1987). The RT-PCR reaction was carried out in a single tube using recombinant *Tth* DNA-polymerase which is capable of reverse transcriptase activity under appropriate reaction condition. The primers used in these experiments (F1 and R1) are based upon highly conserved regions between hPiT1 and hPiT2 located in putative transmembrane domains in the N-terminal and C-terminal regions of the proteins. The results from these experiments show that the products of *Sph*1 digestion are 1000, 487 and 138 bp, which agree with the predicted sizes. The gel patterns under the PiT-1 control after *Sph*I digestion are the same as for K562, after *Sph*I digestion; and both are different from those predicted for PiT-2 after *Sph*I digestion. Thus K562 cells have the human PiT-1 isoform.

EXAMPLE 4

SYNTHESIS OF mRNA FOR CELL-FREE TRANSLATION AND XENOPUS OOCYTE-INJECTION EXPERIMENTS.

The template for expression of the Na-$PO_4$ cotransporter in the cell-free system or in *Xenopus* oocytes is capped mRNA prepared polymerase, prepared by in vitro transcription of linearized cDNA constructs containing the promoter for T7 RNA polymerase. Samples of mRNAs are prepared as prepared as previously described in a 1 ml reaction mixture [Fletcher, L. et al., *J. Biol. Chem.*265;19582(1990)]. These reaction conditions permit high yields of mRNA (~1000 mg or about 100-200 copies of RNA transcript per copy of template DNA) that are >80% capped and migrate as a single discrete band of the correct molecular weight on denaturing PAGE. T7 RNA polymerase is purified as described by Davanloo, et al. *Proc.*

EXAMPLE 5

CELL-FREE EXPRESSION OF NA-$PO_4$ COTRANSPORTER

The Na-$PO_4$ cotransporter mRNA is expressed in a wheat germ cell-free system containing *in vitro* transcribed mRNA, dog pancreatic microsomes and signal recognition particle (SRP). Cell-free translation is carried out in the presence of 34 mM [$^{14}$C]leucine (50 cpm/pmol), 50 mM each of the other 19 amino acids, unfractionated wheat germ tRNA and other components a previously described [Erickson, A.H et al., *Methods Enzymol.* 96;38 (1983)].

Assuming each Na-$PO_4$ cotransporter polypeptide contains~75 leucine (hPiT-1 has 71), synthesis under these conditions yields Na-$PO_4$ cotransporter with a specific activity of ~3750 cpm/pmol. Wheat germ extract is prepared as described by Lax, S.R. et al *Methods Enzymol.* 118;109 (1986), and dog pancreatic microsomes and SRP are prepared according to Walter, P. et al., *Methods Enzymol.* 96; 84-93, & 682-691(1983). Under the appropriate conditions the wheat germ system provides excellent activity, e.g. 5 5 mol ß-globin polypeptide is synthesized per mol ß-globin mRNA template per hour. These data indicate that a 1 ml reaction containing 100 pmol mRNA synthesizes up to 50 mg Na-$PO_4$ cotransporter protein (or nearly 500 pmol) per hour. The synthesis of Na-$PO_4$ cotransporter is monitored by SDS-PAGE and fluorography to determine that the correct molecular weight polypeptide is produced. Translocation and glycosylation is assessed by endogycosidase H and endoglycosidase F treatment. Treated and untreated samples are analyzed by SDS-PAGE.

EXAMPLE 6

growth and selection of transformants are performed by well known techniques. The expression of the Na-$PO_4$ cotransporter is assessed by immunoprecipitation with Na-$PO_4$- specific antibodies of the protein from cells (*Dictyostelium*, HEK 293, etc.) grown in medium supplemented with $^{35}$S-methionine. The functional expression of the Na-$PO_4$ transporter, both native and mutant forms, in transfected cells is monitored by determining the Na-dependent $^{32}$Na-$PO_4$ flux , as described in an Example below. Negative controls include determining background levels of Na-dependent Na-$PO_4$ transport from cells transfected with the construct in the anti-sense orientation. The two principal expression systems for heterologous expression of the erythrocyte Na-$PO_4$ cotransporter are injected *Xenopus* oocytes and transfected *Dictostelium*. Alternatively or additionally, the cotransporter is expressed in another expression system such as HEK 293 or baculovirus-infected Sf9 cells.

A. Injected *Xenopus* oocytes. Stage V and IV oocytes are removed using standard anesthetic (0.17% 3-aminobenzoic acid) and surgical procedures. The oocytes are placed in OR-2 medium and collagenase treated (2mg/ml) for 2.5 h. Individual oocytes are washed and defolliculated if needed by trituration and co-injected with 2.5 ng capped SEAP cRNA and 5-50 ng of capped transporter of cRNA (prepared as described above). Capped SEAP cRNA prepared by *in vitro* transcription of *HInd*III linearized pGEM-SEAP. The pGEM-SEAP construct contains the human placental alkaline phosphatase with a site-specific mutation at codon 489 to create a termination codon[Tate, S.S. et al., *FASEB J.* 4; 228 (1990)]. This stop codon results in a secreted form of alkaline phosphatase rather than a membrane anchored form. cRNAs are injected in a total volume of 50 nl using a Narishige injector. Following incubation overnight in Barth's medium, oocytes are sorted and placed in single wells of a 96 well plate containing 200 ul Barth's medium. Five hours after the oocytes are placed into individual wells, 50 ul of medium is removed for SEAP activity assay. Alkaline phosphatase activity is measured by chromogenic assay. The

secreted alkaline phosphatase catalyzes the dephosporylation of nicotinamide adenine dinucleotide phosphate ($NADP^+$); the NAD formed then catalytically activates an $NAD^+$-specific oxidation-reduction cycle driven by the enzymes alcohol dehydrogenase and diaphorase. The chromophore formed is a violet colored formazan product of INT-violet. Only those oocytes that express SEAP (10 units activity/50 ul at 29 h post-injection) are used in the flux measurements. There is substantial correlation between the level of SEAP activity detected at 29 h post-injection and the level of $^{36}Cl$ flux in oocytes co-injected with SEAP and human AE1or the level of Na-activated $^{32}PO_4$ influx in oocytes injected with SEAP and mRNA for PiT-1, PiT-2, or BNPI. Those oocytes that are positive for SEAP expression are incubated for an additional 1-5 days with daily changes in Barth's medium before influx assays are carried out.

B. *Dictyostelium*. A second expression system for heterologous expression of the cloned cotransporter is *Dictyostelium*. A significant advantage of the *Dictyostelium* expression system is that these cells are grown in suspension culture and are handled like red cells for flux measurements. The principle expression vector (e.g. pBS18 and its derivatives) is based upon selection using the Tn5 gene (neomycin phosphotransferase II) driven by the actin 6 promoter. The insert of interest is driven by the actin 8 promoter and the 2H3 transcriptional terminator. The Tn5 gene permits selection of permanent transfectants in media containing G-418, to which the native slime mold is highly sensitive.

C. HEK-239 cells. Heterologus expression is also readily carried out with HEK-293 cells, ATCC Accession No. CRL 1573. HEK-293 (human embryonic kidney) cells were obtained from the American Type Culture Collection

(ATCC) at passage 31. These cells were used to prepare seed stocks at passage 32. Cells were used until passage 45, after which fresh cultures were started from frozen passage 32 cells. The cells were grown in Minimal Essential Media (MEM) with Hank's salts and supplemented with L-glutamine and 5% fetal calf serum at 37°C in 5% $CO_2$ / 95% air. Transfection was carried out using standard calcium phosphate precipitation methods. Specifically, five days before the transfection, cells were plated in T75 flasks (75 $cm^2$) at 2.5 x $10^4$ / $cm^2$. On the day of the transfection, the cell density was usually 2-3 x $10^5$ / $cm^2$. The cells were washed and fresh media (20 mL) was placed in each culture flask. A 1.0 mL suspension containing the calcium phosphate - DNA precipitate from 40 µg of plasmid DNA was added drop-wise with mixing to the media overlaying the cells. The cells were returned to the incubator for 4 h, then 2mL of 18% (v/v) glycerol was added to the media ("glycerol shocked"), and the cells incubated for an additional two minutes at room temperature. The media was then quickly aspirated from the flask, the cells washed one time with 25 mL Dulbecco's phosphate-buffered saline, fresh media added to the cells (25 mL) and the cells were incubated overnight. The next morning the cells were trypsinized by standard methods, resuspended to a final density of 1.5 - 1.7 x $10^5$ and 1.0 mL of the cell suspension was used to replate the cells in 24-well plates (16 mm diameter wells) at a density of 8.0 x $10^4$ cells/$cm^2$ (1.6 x $10^5$ cells / well). Flux measurements were carried out at 48 ± 6 hr post transfection.

EXAMPLE 7

FLUX MEASUREMENTS

A. Flux measurements in cell-free expression system. The flux is measured by an adaptation of a rapid filtration method according to Macintyre, J.D. et al. *Biochim. Biophys. Acta* 644; 351 (1981). Briefly, microsomes are suspended, equilibrated and mixed in media containing either sodium or choline or N-methyl-alpha-

glucamine as the dominant cation in a thermostatically controlled chamber. The flux is initiated by additional of $^{32}PO_4$ to the flux medium. Aliquots are removed at different times and filtered under vacuum using prewashed mixed cellulose-ester filters. The microsomes retained by the filter are rapidly washed with stopping solution containing 323 mM $MgSO_4$ (isotonic for microsomes). A sample of flux suspension is used to measure total protein and specific activity. The flux (pmol $PO_4$/ug protein-min or ions/cotransporter molecule-min) is calculated from the slope of cpm/aliquot versus time. Preloaded microsomes are used to verify the quantitative recovery of microsomes, the replication of sample counts, and the effectiveness of the wash in removing a extracellular marker, usually $^{14}C$-PEG at 0°C. The probable $^{32}PO_4$ influxes into microsomes are calculated assuming a single copy of the Na-$PO_4$ cotransporter in each 0.05 um microsome, and using kinetic data from erythrocytes, assuming that there are 450 copies of the cotransporter per red blood cell. These calculations indicate that the half-times will be >10 h and are therefore measured by this technique ($t_{½}$ > 5 sec).

B. Flux measurements in oocytes. Oocytes are prepared and injected as described in Example above. Briefly, eight to ten oocytes are placed in individual wells of a 96-well culture plate in medium containing either Na or choline as the dominant cation. The flux is initiated by addition of $^{32}PO_4$ to each well. As known times, oocytes are removed and washed three times in ice cold choline medium. The oocyte is then dissolved in 0.2 ml 10% SDS and the counted in a liquid scintillation counter. A sample of the $^{32}PO_4$ incubation fluid is counted to calculate the extracellular specific activity. The influx (pmol/oocyte/hr) is calculated from the specific activity and the uptake. The difference between the flux in Na and choline media is the calculated Na-dependent phosphate influx.

C. Flux measurements in Dictyostelium discoideum. HL-5 medium contains 20 ± 3 mM K. This is not a defined medium so the composition must be determined for each flux. Cells are grown to a density of 1-3 x $10^6$/ml at 20°C in a shaking incubator. Approximately $10^7$ cells are required for each data point on the influx curve. The cells are resuspended to 4 x $10^7$/ml in HL-5 in a thermostatted stirred chamber at a known pH. At time zero tracer (~0.6 μCi/ml) is added and at known times thereafter samples (0.4 ml) are removed. The samples are transferred to 7 ml of ice-cold stop solution (58 mM $MgSO_4$), immediately centrifuged for 30 seconds at 3000 x g in a rotor, the supernatant aspirated and discarded as radioactive waste. The pellet of cells is resuspended thrice in 6 ml of stop solution, pelleted, and the supernatant aspirated. To the drained pellets 1 ml of 0.1% DOC in 1 N NaOH is added for solubilization. Aliquots are counted for radioactivity or are assayed for protein. The data are calculated as pmoles/mg protein and the data vs. time are fitted to a single exponential by nonlinear regression analysis and the inital slope (flux) and asymptote value constant (pmoles/mg protein) calculated.

D. Flux measurements in cultured mammaliam cells. Human embryonic kidney cells (HEK-293) are purchased from the American Type Culture Collection (ATCC, accession number CRL 1573) at passage 32 and grown in MEM and 5% fetal calf serum in a 5% $CO_2$/95% air 37°C incubator. They are maintained in T75 flasks and split weekly. At passage 45 decreased expression is observed, so frozen stocks at passage 34 are brought up. The cells are transfected with cDNA harvested from bacteria and purified on an anion exchange column. At 24 hours the cells are plated onto 24-well plates at 3-5 x $10^5$ cells/$cm^2$ and transport measured at 48-72 hr. The expression is low by 96 hr and absent at 5 and 10 days. Usually the medium is aspirated and washed once with 0.5 ml of a Na-free (143 mM N-methyl-D-glucamine Cl) $HCO_3$-free MEM-like HEPES buffered medium for 3-5 min. Then it

$^{32}PO_4$ or $^{22}Na$ containing media. Cells transfected with the vector only (e.g., pRBG4) are always treated and fluxed in parallel. $^{32}PO_4$ influx in the absence of Na is always measured. All fluxes are performed in duplicate. The plates are placed on a water thermostatted table for 5 minutes and the flux initiated by aspirating the preincubation medium from the last column of wells and adding the tracer solution at known times (± 0.2 sec.). This is done to successive columns of cells at approximately 30 minutes, 15 minutes, 10 minutes, 5 minutes, 2 minutes and 1 minute prior to terminating the influx simultaneously for all wells on the plate by 3 rapid ice cold washes (over 15 seconds total elapsed time) with (mM): 150 NaCl, 1.5 $CaCl_2$, 1 $MgCl_2$ solution. Residual wash solution is aspirated and the cells in the dry wells are solubilized in 0.5 ml of 25 mM NaOH with 0.5% deoxycholate. A 50 µl sample from each well is used to measure protein and 400 µl sample is counted in a liquid scintillation counter. Quadruplicate 10 µl samples of the influx solution are counted contemporaneously with the flux samples for specific activity determination. The pmol/µg protein in each well is calculated and the slope of the linear least squares best fit to these values against sample times is the computed flux, according to Sarkadi, B. et al., *J.Gen.Physiol.*72: 249 (1978). Usually 5 or 6 of the data points are used to calculate each flux. Each condition is always measured in duplicate in both vector-only transfected and vector+insert transfected cells.

EXAMPLE 8

ISOLATION ,CLONING, and SEQUENCING of LLITHIUM-SODIUM COUNTERTRANSPORTERS.

The hPiT-1 DNA was isolated, cloned and sequenced according to 5,414,076, herein incorporated by reference for this purpose. It is set forth as SEQUENCE ID NO :1 and SEQUENCE ID NO :2

The hPiT-2 DNA was isolated, cloned and sequenced according to 5,550,221, herein incorporated by reference for this purpose. It is set forth as SEQUENCE ID NO.:3 and SEQUENCE ID NO.:4.

The BNPI DNA was isolated, cloned and sequenced according to Ni, B et al., *J.Neurochem.*66, 2227 (1996), herein incorporated by reference for this purpose. It is set forth as SEQUENCE ID NO.:5 and SEQUENCE ID NO.:6.

EXAMPLE 9

PREPARATION OF ANTIBODIES SPECIFIC FOR THE ERYTHROCYTE NA-$PO_4$ COTRANSPORTER.

Polyclonal antibodies are prepared according to England, B.J. et al., *Biochim.Biophys.Acta* 623: 171 (1980), and Timmer, R.T. et al., *J.Biol.Chem.* 268: 24863 (1993). Monoclonal antibodies are prepared according to Kohler, G. et al., *Nature* 256: 495 (1975).

EXAMPLE 10

RESTRCITION LENGTH FRAGMENT POLYMORPHISM ANALYSIS

A. Using primers for hPiT-1, for example,

CAGTTCAGTC AAGCCGTCAG (SEQ ID NO: 7) and

CCAGCCAACA GACACAACAG (SEQ ID NO: 8),

the hPiT-1 sequence is amplified by PCR and ASO, by the methods of Connor, B.J.et al., *Proc.Natl.Acad.Sci.* 80: 278 (1983), and Saiki, R.K. et al., *Nature* 324: 163 (1986). Subsequent digestion with *Taq*I, *Pvu*I, *Mbo*I, and *Sac*I restriction endouncleases is performed.

the hPiT-2 sequence is amplified by PCR and ASO, by the methods of Connor, B.J.et al., *Proc.Natl.Acad.Sci.* 80: 278 (1983), and Saiki, R.K. et al., *Nature* 324: 163 (1986). Subsequent digestion with *Taq*I, *Pvu*I, *Mbo*I, and *Sac*I restriction endouncleases is performed.

C. Using primers for BNPI, for example,
CCTCGCCGCT ACATTATCGC (SEQ ID NO: 11) and
CGAAGCCTCC GCAGTTCATC (SEQ ID NO: 12),
the BNPI sequence is amplified by PCR and ASO, by the methods of Connor, B.J.et al., *Proc.Natl.Acad.Sci.* 80: 278 (1983), and Saiki, R.K. et al., *Nature* 324: 163 (1986). Subsequent digestion with *Taq*I, *Pvu*I, *Mbo*I, and *Sac*I restriction endouncleases is performed.

While the foregoing specification teaches the principles of the present invention, with examples provided for the purpose of illustration, it will be understood that the practice of the invention encompasses all of the usual variations, adaptations, modifications or deletions as come within the scope of the following claims and its equivalents.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: EMORY UNIVERSITY
Gunn, Robert B.
Timmer, Richard T.

(ii) TITLE OF INVENTION: SODIUM-PHOSPHATE COTRANSPORTER IN LITHIUM THERAPY FOR THE TREATMENT OF MENTAL ILLNESS

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Kilpatrick Stockton LLP
(B) STREET: Suite 2800, 1100 Peachtree Street NE
(C) CITY: Atlanta
(D) STATE: GA
(E) COUNTRY: USA
(F) ZIP: 30309-4530

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US98/
(B) FILING DATE: 11-FEB-1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA
(A) APPLICATION NUMBER: US 60/039,462
(B) FILING DATE: 27-FEB-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Meredith, Roy D.
(B) REGISTRATION NUMBER: 30,777
(C) REFERENCE/DOCKET NUMBER: EMU153PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (404) 815-6500
(B) TELEFAX: (404) 815-6555

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 679 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(viii) POSITION IN GENOME:
(B) MAP POSITION: 2q11-q14

(ix) FEATURE:
(A) NAME/KEY: hPIT-1
(B) LOCATION: 1..679
(D) OTHER INFORMATION: /label= Receptor1
/note= "/organism=Homosapiens/cell_line=HL60
/tissue_lib=lambda HGR6, 7, and 16; Clontech
#1020b/map=2q11-q14"

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5,414,076 P

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: O'Hara, B., et al.,
"Characterization of
Human Gene Conferring
Sensitivity to Infection by
Gibbon Ape Leukemia Virus," Cell Growth
Differ. 1(3):119-127 (1990)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ala Thr Leu Ile Thr Ser Thr Thr Ala Ala Thr Ala Ala Ser Gly
1 5 10 15

Pro Leu Val Asp Tyr Leu Trp Met Leu Ile Leu Gly Phe Ile Ile Ala
20 25 30

Phe Val Leu Ala Phe Ser Val Gly Ala Asn Asp Val Ala Asn Ser Phe
35 40 45

Gly Thr Ala Val Gly Ser Gly Val Val Thr Leu Lys Gln Ala Cys Ile
50 55 60

Leu Ala Ser Ile Phe Glu Thr Val Gly Ser Val Leu Leu Gly Ala Lys
65 70 75 80

Val Ser Glu Thr Ile Arg Lys Gly Leu Ile Asp Val Glu Met Tyr Asn
85 90 95

Ser Thr Gln Gly Leu Leu Met Ala Gly Ser Val Ser Ala Met Phe Gly
100 105 110

Ser Ala Val Trp Gln Leu Val Ala Ser Phe Leu Lys Leu Pro Ile Ser
115 120 125

Gly Thr His Cys Ile Val Gly Ala Thr Ile Gly Phe Ser Leu Val Ala

Phe Cys Ala Leu Ile Val Trp Phe Phe Val Cys Pro Arg Met Lys Arg
245 250 255

Lys Ile Glu Arg Glu Ile Lys Cys Ser Pro Ser Glu Ser Pro Leu Met
260 265 270

Glu Lys Lys Asn Ser Leu Lys Glu Asp His Glu Glu Thr Lys Leu Ser
275 280 285

Val Gly Asp Ile Glu Asn Lys His Pro Val Ser Glu Val Gly Pro Ala
290 295 300

Thr Val Pro Leu Gln Ala Val Val Glu Glu Arg Thr Val Ser Phe Lys
305 310 315 320

Leu Gly Asp Leu Glu Glu Ala Pro Glu Arg Glu Arg Leu Pro Ser Val
325 330 335

Asp Leu Lys Glu Glu Thr Ser Ile Asp Ser Thr Val Asn Gly Ala Val
340 345 350

Gln Leu Pro Asn Gly Asn Leu Val Gln Phe Ser Gln Ala Val Ser Asn
355 360 365

Gln Ile Asn Ser Ser Gly His Ser Gln Tyr His Thr Val His Lys Asp
370 375 380

Ser Gly Leu Tyr Lys Glu Leu Leu His Lys Leu His Leu Ala Lys Val
385 390 395 400

Gly Asp Cys Met Gly Asp Ser Gly Asp Lys Pro Leu Arg Arg Asn Asn
405 410 415

Ser Tyr Thr Ser Tyr Thr Met Ala Ile Cys Gly Met Pro Leu Asp Ser
420 425 430

Phe Arg Ala Lys Glu Gly Glu Gln Lys Gly Glu Glu Met Glu Lys Leu
435 440 445

Thr Trp Pro Asn Ala Asp Ser Lys Lys Arg Ile Arg Met Asp Ser Tyr
450 455 460

Thr Ser Tyr Cys Asn Ala Val Ser Asp Leu His Ser Ala Ser Glu Ile

Asp Met Ser Val Lys Ala Ala Met Gly Leu Gly Asp Arg Lys Gly Ser
485 490 495

Asn Gly Ser Leu Glu Glu Trp Tyr Asp Gln Asp Lys Pro Glu Val Ser
500 505 510

Leu Leu Phe Gln Phe Leu Gln Ile Leu Thr Ala Cys Phe Gly Ser Phe
515 520 525

Ala His Gly Gly Asn Asp Val Ser Asn Ala Ile Gly Pro Leu Val Ala
530 535 540

Leu Tyr Leu Val Tyr Asp Thr Gly Asp Val Ser Ser Lys Val Ala Thr
545 550 555 560

Pro Ile Trp Leu Leu Leu Tyr Gly Gly Val Gly Ile Cys Val Gly Leu
565 570 575

Trp Val Trp Gly Arg Arg Val Ile Gln Thr Met Gly Lys Asp Leu Thr
580 585 590

Pro Ile Thr Pro Ser Ser Gly Phe Ser Ile Glu Leu Ala Ser Ala Leu
595 600 605

Thr Val Val Ile Ala Ser Asn Ile Gly Leu Pro Ile Ser Thr Thr His
610 615 620

Cys Lys Val Gly Ser Val Val Ser Val Gly Trp Leu Arg Ser Lys Lys
625 630 635 640

Ala Val Asp Trp Arg Leu Phe Arg Asn Ile Phe Met Ala Trp Phe Val
645 650 655

Thr Val Pro Ile Ser Gly Val Ile Ser Ala Ala Ile Met Ala Ile Phe
660 665 670

Arg Tyr Val Ile Leu Arg Met
675

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3220 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: hPit-1
(B) LOCATION: 1..3220
(D) OTHER INFORMATION: /product= "Leukemia Virus Receptor 1"

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5,414,076 P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAGCTGTCCC CGGTGCCGCC GACCCGGGCC GTGCCGTGTG CCCGTGGCTC CAGCCGCTGC 60

CGCCTCGATC TCCTCGTCTC CCGCTCCGCC CTCCCTTTTC CCTGGATGAA CTTGCGTCCT 120

TTCTCTTCTC CGCCATGGAA TTCTGCTCCG TGCTTTTAGC CCTCCTGAGC CAAAGAAACC 180

CCAGACAACA GATGCCCATA CGCAGCGTAT AGCAGTAACT CCCCAGCTCG GTTTCTGTGC 240

CGTAGTTTAC AGTATTTAAT TTTATATAAT ATATATTATT TATTATAGCA TTTTTGATAC 300

CTCATATTCT GTTTACACAT CTTGAAAGGC GCTCAGTAGT TCTCTTACTA AACAACCACT 360

ACTCCAGAGA ATGGCAACGC TGATTACCAG TACTACAGCT GCTACCGCCG CTTCTGGTCC 420

TTTGGTGGAC TACCTATGGA TGCTCATCCT GGGCTTCATT ATTGCATTTG TCTTGGCATT 480

CTCCGTGGGA GCCAATGATG TAGCAAATTC TTTTGGTACA GCTGTGGGCT CAGGTGTAGT 540

GACCCTGAAG CAAGCCTGCA TCCTAGCTAG CATCTTTGAA ACAGTGGGCT CTGTCTTACT 600

GGGGGCCAAA GTGAGCGAAA CCATCCGGAA GGGCTTGATT GACGTGGAGA TGTACAACTC 660

GACTCAAGGG CTACTGATGG CCGGCTCAGT CAGTGCTATG TTTGGTTCTG CTGTGTGGCA 720

ACTCGTGGCT TCGTTTTTGA AGCTCCCTAT TTCTGGAACC CATTGTATTG TTGGTGCAAC 780

CTTCCTGGTT CGTGCATTCA TCCTCCATAA GGCAGATCCA GTTCCTAATG GTTTGCGAGC 960

TTTGCCAGTT TTCTATGCCT GCACAGTTGG AATAAACCTC TTTTCCATCA TGTATACTGG 1020

AGCACCGTTG CTGGGCTTTG ACAAACTTCC TCTGTGGGGT ACCATCCTCA TCTCGGTGGG 1080

ATGTGCAGTT TTCTGTGCCC TTATCGTCTG GTTCTTTGTA TGTCCCAGGA TGAAGAGAAA 1140

AATTGAACGA GAAATAAAGT GTAGTCCTTC TGAAAGCCCC TTAATGGAAA AAAAGAATAG 1200

CTTGAAAGAA GACCATGAAG AAACAAAGTT GTCTGTTGGT GATATTGAAA ACAAGCATCC 1260

TGTTTCTGAG GTAGGGCCTG CCACTGTGCC CCTCCAGGCT GTGGTGGAGG AGAGAACAGT 1320

CTCATTCAAA CTTGGAGATT TGGAGGAAGC TCCAGAGAGA GAGAGGCTTC CCAGCGTGGA 1380

CTTGAAAGAG GAAACCAGCA TAGATAGCAC CGTGAATGGT GCAGTGCAGT TGCCTAATGG 1440

GAACCTTGTC CAGTTCAGTC AAGCCGTCAG CAACCAAATA AACTCCAGTG GCCACTCCCA 1500

GTATCACACC GTGCATAAGG ATTCCGGCCT GTACAAAGAG CTACTCCATA AATTACATCT 1560

TGCCAAGGTG GGAGATTGCA TGGGAGACTC CGGTGACAAA CCCTTAAGGC GCAATAATAG 1620

CTATACTTCC TATACCATGG CAATATGTGG CATGCCTCTG GATTCATTCC GTGCCAAAGA 1680

AGGTGAACAG AAGGGCGAAG AAATGGAGAA GCTGACATGG CCTAATGCAG ACTCCAAGAA 1740

GCGAATTCGA ATGGACAGTT ACACCAGTTA CTGCAATGCT GTGTCTGACC TTCACTCAGC 1800

ATCTGAGATA GACATGAGTG TCAAGGCAGC GATGGGTCTA GGTGACAGAA AAGGAAGTAA 1860

CCTTCCCATC AGTACAACAC ATTGTAAAGT GGGCTCTGTT GTGTCTGTTG GCTGGCTCCG 2280

GTCCAAGAAG GCTGTTGACT GGCGTCTCTT TCGTAACATT TTTATGGCCT GGTTTGTCAC 2340

AGTCCCCATT TCTGGAGTTA TCAGTGCTGC CATCATGGCA ATCTTCAGAT ATGTCATCCT 2400

CAGAATGTGA AGCTGTTTGA GATTAAAATT TGTGTCAATG TTTGGGACCA TCTTAGGTAT 2460

TCCTGCTCCC CTGAAGAATG ATTACAGTGT TAACAGAAGA CTGACAAGAG TCTTTTTATT 2520

TGGGAGCAGA GGAGGGAAGT GTTACTTGTG CTATAACTGC TTTTGTGCTA AATATGAATT 2580

GTCTCAAAAT TAGCTGTGTA AAATAGCCCG GGTTCCACTG GCTCCTGCTG AGGTCCCCTT 2640

TCCTTCTGGG CTGTGAATTC CTGTACATAT TTCTCTACTT TTTGTATCAG GCTTCAATTC 2700

CATTATGTTT TAATGTTGTC TCTGAAGATG ACTTGTGATT TTTTTTTCTT TTTTTTAAAC 2760

CATGAAGAGC CGTTTGACAG AGCATGCTCT GCGTTGTTGG TTTCACCAGC TTCTGCCCTC 2820

ACATGCACAG GGATTTAACA ACAAAAATAT AACTACAACT TCCCTTGTAG TCTCTTATAT 2880

AAGTAGAGTC CTTGGTACTC TGCCCTCCTG TCAGTAGTGG CAGGATCTAT TGGCATATTC 2940

GGGAGCTTCT TAGAGGGATG AGGTTCTTTG AACACAGTGA AAATTTAAAT TAGTAACTTT 3000

TTTGCAAGCA GTTTATTGAC TGTTATTGCT AAGAAGAAGT AAGAAAGAAA AAGCCTGTTG 3060

GCAATCTTGG TTATTTCTTT AAGATTTCTG GCAGTGTGGG ATGGATGAAT GAAGTGGAAT 3120

GTGAACTTTG GGCAAGTTAA ATGGGACAGC CTTCCATGTT CATTTGTCTA CCTCTTAACT 3180

GAATAAAAAA GCCTACAGTT TTTAGAAAAA ACCCGAATTC 3220

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 652 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: placenta

(ix) FEATURE:
(A) NAME/KEY: hPiT-2
(B) LOCATION: 1..652
(D) OTHER INFORMATION: /label= Receptor2
/note= "/organism=homo sapiens /sex=male
/tissue_type=placenta
tissue_lib=Stratagene #936203"

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: van Zeijl, M., et al., "A Human Amphotropic Retrovirus Receptor is a Second Member of the Gibbon Ape Leukemia Virus Receptor Family," Proc. Natl. Acad. Sci., U.S.A., 91(3):1168-1172 (1994)

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5,550,221 P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ala Met Asp Glu Tyr Leu Trp Met Val Ile Leu Gly Phe Ile Ile
1 5 10 15

Ala Phe Ile Leu Ala Phe Ser Val Gly Ala Asn Asp Val Ala Asn Ser
20 25 30

Phe Gly Thr Ala Val Gly Ser Gly Val Val Thr Leu Arg Gln Ala Cys
35 40 45

Ile Leu Ala Ser Ile Phe Glu Thr Thr Gly Ser Val Leu Leu Gly Ala
50 55 60

Lys Val Gly Glu Thr Ile Arg Lys Gly Ile Ile Asp Val Asn Leu Tyr
65 70 75 80

Asn Glu Thr Val Glu Thr Leu Met Ala Gly Glu Val Ser Ala Met Val
85 90 95

Gly Ser Ala Val Trp Gln Leu Ile Ala Ser Phe Leu Arg Leu Pro Ile
100 105 110

Ser Gly Thr His Cys Ile Val Gly Ser Thr Ile Gly Phe Ser Leu Val
115 120 125

Ala Ile Gly Thr Lys Gly Val Gln Trp Met Glu Leu Val Lys Ile Val
130 135 140

Gly Ala Ala Gly Glu Thr Leu Gly Thr Ser Glu Gly Thr Ser Ala Gly
290 295 300

Ser His Pro Arg Ala Ala Tyr Gly Arg Ala Leu Ser Met Thr His Gly
305 310 315 320

Ser Val Lys Ser Pro Ile Ser Asn Gly Thr Phe Gly Phe Asp Gly His
325 330 335

Thr Arg Ser Asp Gly His Val Tyr His Thr Val His Lys Asp Ser Gly
340 345 350

Leu Tyr Lys Asp Leu Leu His Lys Ile His Ile Asp Arg Gly Pro Glu
355 360 365

Glu Lys Pro Ala Gln Glu Ser Asn Tyr Arg Leu Leu Arg Arg Asn Asn
370 375 380

Ser Tyr Thr Cys Tyr Thr Ala Ala Ile Cys Gly Leu Pro Val His Ala
385 390 395 400

Thr Phe Arg Ala Ala Asp Ser Ser Ala Pro Glu Asp Ser Glu Lys Leu
405 410 415

Val Gly Asp Thr Val Ser Tyr Ser Lys Lys Arg Leu Arg Tyr Asp Ser
420 425 430

Tyr Ser Ser Tyr Cys Asn Ala Val Ala Glu Ala Glu Ile Glu Ala Glu
435 440 445

Glu Gly Gly Val Glu Met Lys Leu Ala Ser Glu Leu Ala Asp Pro Asp
450 455 460

Gln Pro Arg Glu Asp Pro Ala Glu Glu Glu Lys Glu Glu Lys Asp Ala
465 470 475 480

Pro Glu Val His Leu Leu Phe His Phe Leu Gln Val Leu Thr Ala Cys
485 490 495

Phe Gly Ser Phe Ala His Gly Gly Asn Asp Val Ser Asn Ala Ile Gly
500 505 510

Pro Leu Val Ala Leu Trp Leu Ile Tyr Lys Gln Gly Gly Val Thr Gln
515 520 525

Glu Ala Ala Thr Pro Val Trp Leu Leu Phe Tyr Gly Gly Val Gly Ile
530 535 540

Cys Thr Gly Leu Trp Val Trp Gly Arg Arg Val Ile Gln Thr Met Gly
545 550 555 560

Lys Asp Leu Thr Pro Ile Thr Pro Ser Ser Gly Phe Thr Ile Glu Leu
565 570 575

Ala Ser Ala Phe Thr Val Val Ile Ala Ser Asn Ile Gly Leu Pro Val
580 585 590

Ser Thr Thr His Cys Lys Val Gly Ser Val Val Ala Val Gly Trp Ile
595 600 605

Arg Ser Arg Lys Ala Val Asp Trp Arg Leu Phe Arg Asn Ile Phe Val
610 615 620

Ala Trp Phe Val Thr Val Pro Val Ala Gly Leu Phe Ser Ala Ala Val
625 630 635 640

Met Ala Leu Leu Met Tyr Gly Ile Leu Pro Tyr Val
645 650

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3175 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(F) TISSUE TYPE: placenta

(ix) FEATURE:
(A) NAME/KEY: hPiT-2
(B) LOCATION: 1..3175
(D) OTHER INFORMATION: /product= "Leukemia virus receptor 2"
/label= Receptor2

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: van Zeijl, M., et al., "A Human Amphotropic Retrovirus Receptor is a Second Member of the Gibbon Ape Leukemia Virus Receptor Family," Proc. Natl. Acad. Sci., U.S.A., 91(3):1168-1172 (1994)

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5,550,221 P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CAGATCGGGA AGAAAAATAT GGAATGTGTT TTACCGCTGA CTGAACACAA CCAAATGAAC 60

TGTCCTGACA GTAGTTTGCA AACCAGCAGC TAGCAGTTTG TCCAGCCTCT AACATTGTCC 120

AGCACTTTCC AGAGCAAACT CACTGTTTAC AAGAACTCTT GGCCTTACGA AGTTTATAAC 180

CTCAAGCTTT GTTTATTTAA AATATTCCTG CAAAAGAAAA GTACCCGGCA CCCACTTTCC 240

AAAATGGCCA TGGATGAGTA TTTGTGGATG GTCATTTTGG GTTTCATCAT AGCTTTCATC 300

TTGGCCTTTT CTGTTGGTGC AAACGATGTT GCCAACTCCT TTGGTACAGC CGTGGGCTCT 360

GGTGTGGTGA CCTTGAGGCA GGCATGCATT TTAGCTTCAA TATTTGAAAC CACCGGCTCC 420

GTGTTACTAG GCGCCAAAGT AGGAGAAACC ATTCGCAAAG GTATCATTGA CGTGAACCTG 480

TACAACGAGA CGGTGGAGAC TCTCATGGCT GGGGAAGTTA GTGCCATGGT TGGTTCCGCT 540

GTGTGGCAGC TGATTGCTTC CTTCCTGAGG CTTCCAATCT CAGGAACGCA CTGCATTGTG 600

GGTTCTACTA TAGGATTCTC ACTGGTCGCA ATCGGTACCA AAGGTGTGCA GTGGATGGAG 660

CTTGTCAAGA TTGTTGCTTC TTGGTTTATA TCTCCACTGT TGTCTGGTTT CATGTCTGGC 720

CTGCTGTTTG TACTCATCAG AATTTTCATC TTAAAAAAGG AAGACCCTGT TCCCAATGGC 780

CTCCGGGCAC TCCCAGTATT CTATGCTGCT ACCATAGCAA TCAATGTCTT TTCCATCATG 840

TACACAGGAG CACCAGTGCT CGGCCTTGTT CTCCCCATGT GGGCCATAGC CCTCATTTCC 900

TTTGGTGTCG CCCTCCTGTT CGCTTTTTTT GTGTGGCTCT TCGTGTGTCC GTGGATGCGG 960

AGGAAAATAA CAGGCAAATT ACAAAAAGAA GGTGCTTTAT CACGAGTATC TGACGAAAGC 1020

CTCAGTAAGG TTCAGGAAGC AGAGTCCCCA GTATTTAAAG AGCTACCAGG TGCCAAGGCT 1080

AATGATGACA GCACCATCCC GCTCACGGGA GCAGCAGGGG AGACACTGGG GACCTCGGAA 1140

GGCACTTCTG CGGGCAGCCA CCCTCGGGCT GCATACGGAA GAGCACTGTC CATGACCCAT 1200

GGCTCTGTGA AATCGCCCAT CTCCAACGGC ACCTTCGGCT TCGACGGCCA CACCAGGAGC 1260

GACGGTCATG TGTACCACAC CGTGCACAAA GACTCGGGGC TCTACAAAGA TCTGCTGCAC 1320

AAAATCCACA TCGACAGGGG CCCCGAGGAG AAGCCAGCCC AGGAAAGCAA CTACCGGCTG 1380

CTCCGCCGAA ACAACAGTTA CACCTGCTAC ACCGCAGCCA TTTGTGGGCT GCCAGTGCAC 1440

GCCACCTTTC GAGCTGCGGA CTCATCGGCC CCAGAGGACA GTGAGAAGCT GGTGGGCGAC 1500

ACCGTGTCCT ACTCCAAGAA GAGGCTGCGC TACGACAGCT ACTCGAGCTA CTGTAACGCG 1560

GTGGCAGAGG CGGAGATCGA GGCGGAGGAG GGCGGCGTGG AGATGAAGCT GGCGTCGGAG 1620

CTGGCCGACC CTGACCAGCC GCGAGAGGAC CCTGCAGAGG AGGAGAAGGA GGAGAAGGAC 1680

GCACCCGAGG TTCACCTCCT GTTCCATTTC CTGCAGGTCC TCACCGCCTG TTTCGGGTCC 1740

TTTGCTCACG GCGGCAATGA CGTGAGTAAT GCCATCGGTC CCCTGGTAGC CTTGTGGCTG 1800

ATTTACAAAC AAGGCGGGGT AACGCAAGAA GCAGCTACAC CCGTCTGGCT GCTGTTTTAT 1860

TACTGTACAT AATAATATGT ATTAAACTGG TATCGTGGTG ATATAATGTG GTGCAGTTAC 2460

TTATATATTA AATATCTATT GTATCCATAG AATAGGCAGC ATTATTTCAA ACATATTCAA 2520

GTTGGGAGTG GAGATCATTG CCTAGAAGTC AATATTCAAT AAATCTTGTA CATAACTATT 2580

TCGATGGCAA ATGTTAAGCC TTCTAAAAGG AAAGTGTAGA TTGGAAAATG ATTTTTTTTC 2640

CAAATGATGT TTTTGCCTTC TAATATACTG TAAGGTAATG AGCTTCAGAA CAGGCAACCT 2700

GACCCTGCAG AGGTCGCGTG CTGTGGGATG ACAGCGGGAC GGGAGCTCAC AAGTGCTTTC 2760

ACTGAAGATT TGTTCATATA CTGTGTATTG ATTGTTGTGT AATATATCAT CATTGCTTTT 2820

GTAAATACGT AAAACTGTAA TTTTTTAATG GTGTGCTTCC CTTATACTTT TTGATCAGAG 2880

AATTTTGGAA AGTACCAAAG AAGCAGGGGA ATCATTGGCC AGTGTTACGT TTTCACATTG 2940

TCTGTCTCCC ACCCTCACTG ATCACGCCTG CCCCAGAGCA GTGTGTGGCG GTGACACCGT 3000

CACCCAGCAT GCGCCACGCC GTCGTCCCAC CAGCAGTGCC ACCGCCACCA CACCCCAGAT 3060

CCCACCCACC TTGCAGTGGC TTTCTTGTCA TCAGAGTAGA GAATGCACAG GTGTTGGTGA 3120

GGGCGTGTGG CTGAGCACTA CATGTCAAGT CAGAGTCAGT TTCTATCCAA TTCTC 3175

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 560 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: hBNPI
(B) LOCATION: 1..560

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: Ni, B., et al.,
J. Neurochem., 66:2227 (1996)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Glu Phe Arg Gln Glu Glu Phe Arg Lys Leu Ala Gly Arg Ala Leu
1 5 10 15

Gly Lys Leu His Arg Leu Leu Glu Lys Arg Gln Glu Gly Ala Glu Thr
20 25 30

Val Glu Leu Ser Ala Asp Gly Arg Pro Val Thr Thr Gln Thr Arg Asp
35 40 45

Pro Pro Val Val Asp Cys Thr Cys Phe Gly Leu Pro Arg Arg Tyr Ile
50 55 60

Pro Glu Thr Val Gly Leu Ile His Gly Ser Phe Phe Trp Gly Tyr Ile
115 120 125

Val Thr Gln Ile Pro Gly Gly Phe Ile Cys Gln Lys Phe Ala Ala Asn
130 135 140

Arg Val Phe Gly Phe Ala Ile Val Ala Thr Ser Thr Leu Asn Met Leu
145 150 155 160

Ile Pro Ser Ala Ala Arg Val His Tyr Gly Cys Val Ile Phe Val Arg
165 170 175

Ile Leu Gln Gly Leu Val Glu Gly Val Thr Tyr Pro Ala Cys His Gly
180 185 190

Ile Trp Ser Lys Trp Ala Pro Pro Leu Glu Arg Ser Arg Leu Ala Thr
195 200 205

Thr Ala Phe Cys Gly Ser Tyr Ala Gly Ala Val Val Ala Met Pro Leu
210 215 220

Ala Gly Val Leu Val Gln Tyr Ser Gly Trp Ser Ser Val Phe Tyr Val
225 230 235 240

Tyr Gly Ser Phe Gly Ile Phe Trp Tyr Leu Phe Trp Leu Leu Val Ser
245 250 255

Tyr Glu Ser Pro Ala Leu His Pro Ser Ile Ser Glu Glu Glu Arg Lys
260 265 270

Tyr Ile Glu Asp Ala Ile Gly Glu Ser Ala Lys Leu Met Asn Pro Leu
275 280 285

Thr Lys Phe Ser Thr Pro Trp Arg Arg Phe Phe Thr Ser Met Pro Val
290 295 300

Tyr Ala Ile Ile Val Ala Asn Phe Cys Arg Ser Trp Thr Phe Tyr Leu
305 310 315 320

Leu Leu Ile Ser Gln Pro Asp Tyr Phe Glu Glu Val Phe Gly Phe Glu
325 330 335

Ile Ser Lys Val Gly Leu Val Ser Ala Leu Pro His Leu Val Met Thr
340 345 350

Ile Ile Val Pro Ile Gly Gly Gln Ile Ala Asp Phe Leu Arg Ser Arg
355 360 365

Arg Ile Met Ser Thr Thr Asn Val Arg Lys Leu Met Asn Cys Gly Gly
370 375 380

Phe Gly Met Glu Ala Thr Leu Leu Leu Val Val Gly Tyr Ser His Ser
385 390 395 400

Lys Gly Val Ala Ile Ser Phe Leu Val Leu Ala Val Gly Phe Ser Gly
405 410 415

Phe Ala Ile Ser Gly Phe Asn Val Asn His Leu Asp Ile Ala Pro Arg
420 425 430

Tyr Ala Ser Ile Leu Met Gly Ile Ser Asn Gly Val Gly Thr Leu Ser
435 440 445

Gly Met Val Cys Pro Ile Ile Val Gly Ala Met Thr Lys His Lys Thr
450 455 460

Arg Glu Glu Trp Gln Tyr Val Phe Leu Ile Ala Ser Leu Val His Tyr
465 470 475 480

Gly Gly Val Ile Phe Tyr Gly Val Phe Ala Ser Gly Glu Lys Gln Pro
485 490 495

Trp Ala Glu Pro Glu Glu Met Ser Glu Glu Lys Cys Gly Phe Val Gly
500 505 510

His Asp Gln Leu Ala Gly Ser Asp Asp Ser Glu Met Glu Asp Glu Ala
515 520 525

Glu Pro Pro Gly Ala Pro Pro Ala Pro Pro Pro Ser Tyr Gly Ala Thr
530 535 540

His Ser Thr Phe Gln Pro Pro Arg Pro Pro Pro Pro Val Arg Asp Tyr
545 550 555 560

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2716 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: hBNPI
(B) LOCATION: 1..2716

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: Ni, B., et al.,
J. Neurochem., 66:2227 (1996)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGATAAGCTT GATATCGAAT TCCGGACTCT TGCTCGGGCG CCTTAACCCG GCGTTCGGTT 60

CATCCCGCAG CGCCAGTTCT GCTTACCAAA AGTGGCCCAC TAGGCACTCG CATTCCACGC 120

CCGGCTCCAC GCCAGCGAGC CGGGCTTCTT ACCCATTTAA AGTTTGAGAA TAGGTTGAGA 180

TCGTTTCGGC CCCAAGACCT CTAATCATTC GCTTTACCGG ATAAAACTGC GTGGCGGGGG 240

TGCGTCGGGT CTGCGAGAGC GCCAGCTATC CTGAGGGAAA CTTCGGAGGG AACCAGCTAC 300

TAGATGGTTC GATTAGTCTT TCGCCCCTAT ACCCAGGTCG GACGACCGAT TTGCACGTCA 360

GGACCGCTAC GGACCTCCAC CAGAGTTTCC TCTGGCTTCG CCCTGCCCAG GCGATCGGCG 420

GGGGGGACCC GCGGGGTGAC CGGCGGCAGG AGCCGCCACC ATGGAGTTCC GCCAGGAGGA 480

GTTTCGGAAG CTAGCGGGTC GTGCTCTCGG GAAGCTGCAC CGCCTTCTGG AGAAGCGGCA 540

GGAAGGCGCG GAGACGGTGG AGCTGAGTGC GGATGGGCGC CCGGTGACCA CGCAGACCCG 600

GGACCCGCCG GTGGTGGACT GCACCTGCTT CGGCCTCCCT CGCCGCTACA TTATCGCCAT 660

CATGAGTGGT CTGGGCTTCT GCATCAGCTT TGGCATCCGC TGCAACCTGG GCGTGGCCAT 720

CGTCTCCATG GTCAATAACA GCACGACCCA CCGCGGGGGC CACGTGGTGG TGCAGAAAGC 780

CCAGTTCAGC TGGGATCCAG AGACTGTCGG CCTCATACAC GGCTCCTTTT TCTGGGGCTA 840

CATTGTCACT CAGATTCCAG GAGGATTTAT CTGTCAAAAA TTTGCAGCCA ACAGAGTTTT 900

CGGCTTTGCT ATTGTGGCAA CATCCACTCT AAACATGCTG ATCCCCTCAG CTGCCCGCGT 960

CCACTATGGC TGTGTCATCT TCGTGAGGAT CCTGCAGGGG TTGGTAGAGG GGGTCACATA 1020

CCCCGCCTGC CATGGGATCT GGAGCAAATG GGCCCCACCC TTAGAACGGA GTCGCCTGGC 1080

GACGACAGCC TTTTGTGGTT CCTATGCTGG GGCGGTGGTC GCGATGCCCC TCGCCGGGGT 1140

CCTTGTGCAG TACTCAGGAT GGAGCTCTGT TTTCTACGTC TACGGCAGCT TCGGGATCTT 1200

CTGGTACCTG TTCTGGCTGC TCGTCTCCTA CGAGTCCCCC GCGCTGCACC CCAGCATCTC 1260

GGAGGAGGAG CGCAAGTACA TCGAGGACGC CATCGGAGAG AGCGCGAAAC TCATGAACCC 1320

CCTCACGAAG TTTAGCACTC CCTGGCGGCG CTTCTTCACG TCTATGCCAG TCTATGCCAT 1380

CATCGTGGCC AACTTCTGCC GCAGCTGGAC GTTCTACCTG CTGCTCATCT CCCAGCCCGA 1440

CTACTTCGAA GAAGTGTTCG GCTTCGAGAT CAGCAAGGTA GGCCTGGTGT CCGCGCTGCC 1500

CCACCTGGTC ATGACCATCA TCGTGCCCAT CGGCGGCCAG ATCGCGGACT TCCTGCGGAG 1560

CCGCCGCATC ATGTCCACCA CCAACGTGCG CAAGTTGATG AACTGCGGAG GCTTCGGCAT 1620

GGAAGCCACG CTGCTGTTGG TGGTCGGCTA CTCGCACTCC AAGGGCGTGG CCATCTCCTT 1680

CCTGGTCCTA GCCGTGGGCT TCAGCGGCTT CGCCATCTCT GGGTTCAACG TGAACCACCT 1740

GGACATAGCC CCGCGCTACG CCAGCATCCT CATGGGCATC TCCAACGGCG TGGGCACACT 1800

GTCGGGCATG GTGTGCCCCA TCATCGTGGG GGCCATGACT AAGCACAAGA CTCGGGAGGA 1860

GTGGCAGTAC GTGTTCCTAA TTGCCTCCCT GGTGCACTAT GGAGGTGTCA TCTTCTACGG 1920

GGTCTTTGCT TCTGGAGAGA AGCAGCCGTG GGCAGAGCCT GAGGAGATGA GCGAGGAGAA 1980

GTGTGGCTTC GTTGGCCATG ACCAGCTGGC TGGCAGTGAC GACAGCGAAA TGGAGGATGA 2040

GGCTGAGCCC CCGGGGGCAC CCCCTGCACC CCCGCCCTCC TATGGGGCCA CACACAGCAC 2100

ATTTCAGCCC CCCAGGCCCC CACCCCCTGT CCGGGACTAC TGACCATGTG CCTCCCACTG 2160

AATGGCAGTT TCCAGGACCT CCATTCCACT CATCTCTGGC CTGAGTGACA GTGTCAAGGA 2220

ACCCTGCTCC TCTCTGTCCT GCCTCAGGCC TAAGAAGCAC TCTCCCTTGT TCCCAGTGCT 2280

GTCAAATCCT CTTTCCTTCC CAATTGCCTC TCAGGGGTAG TGAAGCTGCA GACTGACAGT 2340

TTCAAGGATA CCCAAATTCC CCTAAAGGTT CCCTCTCCAC CCGTTCTGCC TCAGTGGTTT 2400

CAAATCTCTC CTTTCAGGGC TTTATTTGAA TGGACAGTTC GACCTCTTAC TCTCTCTTGT 2460

GGTTTTGAGG CACCCACACC CCCCGCTTTC CTTTATCTCC AGGGACTCTC AGGCTAACCT 2520

TTGAGATCAC TCAGCTCCCA TCTCCTTTCA GAAAAATTCA AGGTCCTCCT CTAGAAGTTT 2580

CAAATCTCTC CCAACTCTGT TCTGCATCTT CCAGATTGGT TTAACCAATT ACTCGTCCCC 2640

GCCATTCCAG GGATTGATTC TCACCAGCGT TTCTGATGGA AAATGGCGGG AATTCCTGCA 2700

GCCCGGGGGA TCCACT 2716

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: primer for hPiT-1
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGTTCAGTC AAGCCGTCAG 20

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: complementary strand primer for hPiT-1
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCAGCCAACA GACACAACAG 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: primer for hPiT-1
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACAACGAGAC GGTGGAGACT 20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: complementary strand primer for hPiT-2
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGCGGTGTAG CAGGTGTAAC 20

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: primer for hBNPI
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCTCGCCGCT ACATTATCGC 20

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: complementary strand primer for hBNPI
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CGAAGCCTCC GCAGTTCATC 20

WHAT IS CLAIMED IS:

1. A purified DNA molecule coding for a lithium-sodium countertransporter.

2. A purified DNA molecule coding for an amino acid sequence selected from the group consisting of hPiT-1, hPiT-2, and hBNPI, said molecule useful for measuring lithium-sodium countertransport in human cells.

3. The purified DNA molecule of claims 1 or 2, wherein the nucleotide sequence is SEQ.ID.NO.: 2.

4. The purified DNA molecule of claims 1 or 2, said DNA encoding for the amino acid sequence of SEQ.ID.NO.: 1.

5. The purified DNA molecule of claims 1 or 2, wherein the nucleotide sequence is SEQ.ID.NO.: 4.

6. The purified DNA molecule of claims 1 or 2, said DNA encoding for the amino acid sequence of SEQ.ID.NO.: 3.

7. The purified DNA molecule of claims 1 or 2, wherein the nucleotide sequence is SEQ.ID.NO.: 6.

8. The purified DNA molecule of claims 1 or 2, said DNA encoding for the amino acid sequence of SEQ.ID.NO.: 5.

9. A h[illegible] amphotrophic retrovirus receptor useful as a lithium-sodium

10. The receptor of claim 9, wherein its nucleotide sequence is SEQ.ID.NO. 2.

11. The receptor of claim 9, wherein its amino acid sequence is SEQ.ID.NO.: 1.

12. The receptor of claim 9, wherein its nucleotide sequence is SEQ.ID.NO. 4.

13. The receptor of claim 9, wherein its amino acid sequence is SEQ.ID.NO.: 3.

14. The receptor of claim 9, wherein its nucleotide sequence is SEQ.ID.NO. 6.

15. The receptor of claim 9, wherein its amino acid sequence is SEQ.ID.NO.: 5.

16. A method of evaluating sensitivity to lithium therapy in manic depressive patients, comprising the steps of

(a) providing a sample of patient blood;

(b) extracting from the blood sample the patient's DNA;

(c) subjecting the DNA to hybridization with primers specific for any sequence coding for lithium-sodium countertransporter;

(d) polymerizing said sequences, to give polymerized sequences;

(e) amplifying said polymerized sequences, to give an amplified sample of patient sequences;

(f) digesting the amplified sample with one or more restriction endonucleases suitable for mapping sites on the DNA indicating susceptibility to lithium therapy.

17. A method of evaluating sensitivity to lithium therapy in manic depressive patients, comprising the steps of

(a) providing a sample of patient blood;

(b) extracting from the blood sample the patient's DNA;

(c) subjecting the DNA to hybridization with primers specific for any sequence coding for lithium-sodium countertransporter;

(d) polymerizing said sequences, to give polymerized sequences;

(e) amplifying said polymerized sequences, to give an amplified sample of patient sequences;

(f) subjecting the amplified sample to in vitro membrane-based translation to give a translated sample within a cell; and

(g) subjecting the translated sample to flux analysis of lithium, to evaluate sensitivity to lithium therapy in manic depressive patients.

18. The method of claims 16 or 17, wherein the sequence coding for the lithium-sodium countertransporter is selected from the group consisting of hPiT-1, hPiT-2, and hBNPI.

19. The method of claims 16 or 17, wherein the sequence is the nucleotide sequence coding for the lithium-sodium countertransporter selected from the group consisting of SEQ.ID.NO.:2, SEQ.ID.NO.:4,. and SEQ.ID.NO.:6.

20. The method of claims 16 or 17, wherein the sequence is the amino acid sequence for the lithium-sodium countertransporter is selected from the group consisting of SEQ.ID.NO.:1, SEQ.ID.NO.:3,. SEQ.ID.NO.:5.

21. A method of evaluating sensitivity to lithium therapy in manic depressive patients, comprising the steps of

(a) providing a sample of patient blood;

(b) isolating the erythrocytes;

(c) subjecting the erythrocytes to flux analysis of lithium, to evaluate sensitivity to lithium therapy in manic depressive patients.

22. A method of evaluating lithium-sodium countertransport in patients with mental illness, comprising the steps of

(a) providing a sample of patient blood;

(b) isolating the erythrocytes;

(c) subjecting the erythrocytes to flux analysis of lithium, to evaluate lithium-sodium countertransport.

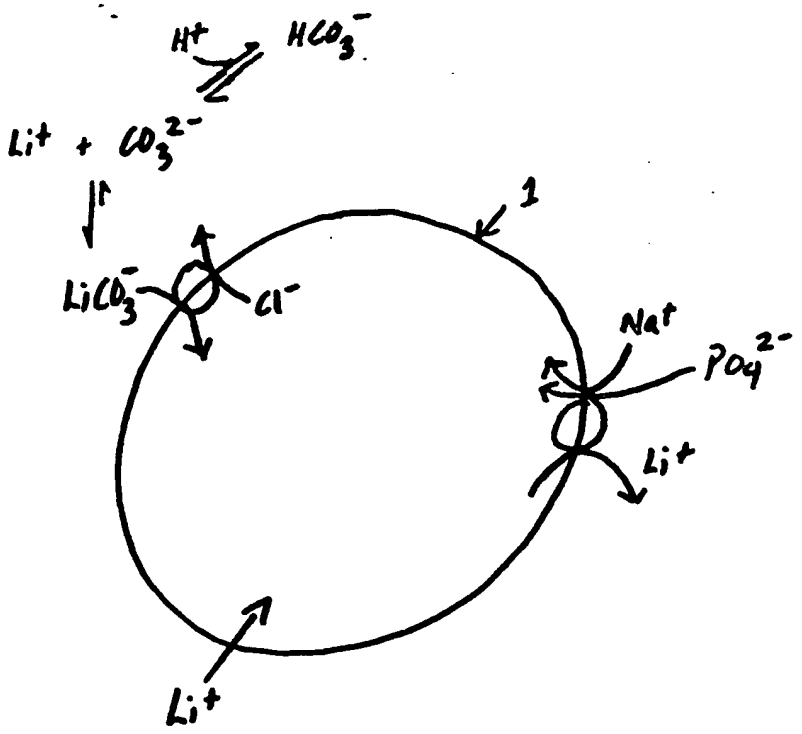


Figure 1

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02875

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : C07H 21/00; G01N 33/50; C07K 14/00
US CL : 536/23.5; 435/7.21; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/7.21; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5,414,076 A (O'HARA) 09 May 1995, SEQ ID NO:1 and 2. | 1-4, 9-11 |
| X | O'HARA, B. et al. Characterization of a Human Gene Conferring Sensitivity to Infection by Gibbon Ape Leukemia Virus. Cell Growth and Differentiation. 1990, Vol. 1, pages 119-127, especially Fig. 3. | 1-4, 9-11 |
| X,P | US 5,633,348 A (JOHANN et al.) 27 May 1997, SEQ ID NO:1 and 2. | 1-2, 5-6, 9, 12-13 |
| X | Us 5,550,221 A (JOHANN et al.) 27 August 1996, SEQ ID NO:1 and 2. | 1-2, 5-6, 9, 12-13 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>12 MAY 1998 | Date of mailing of the international search report<br>19 JUN 1998 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>NIRMAL S. BASI<br>Telephone No. (703) 308-0196 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02875

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ZEIJL, M. et al. A Human Amphotropic Retrovirus Receptor is a Second Member of the Gibbon Ape Leukemia Virus Receptor Family. Proc. Natl. Acad. Sci. 1994, Vol. 91, pages 1168-1172, especially Fig. 1. | 1-2, 6, 9, 11 |
| X | MILLER, G. M. et al. Cloning of the Cellular Receptor for Amphotropic Murine Retroviruses Reveals Homology to that for Gibbon Ape Leukemia Virus. Proc. Natl. Acad. Sci. January 1994, Vol. 91, pages 78-82, especially Fig 1. | 1-2, 6, 9, 11 |
| X,P | US 5,618, 918 A (NI et al.) 08 April 1997, SEQ ID NO:1 and 2. | 1-2, 7-9, 14-15 |
| X,P | US 5,618,677 A (NI et al.) 08 April 1997, SEQ ID NO:1 and 2. | 1-2, 7-9, 14-15 |
| X,P | US 5,686,266 A (NI et al.) 11 November 1997, SEQ ID NO:1 and 2 . | 1-2, 7-7, 14-15 |
| A | NI, B. et al. Cloning and Expression of a cDNA Encoding a Brain-Specific $Na^+$-dependent Inorganic Phosphate Cotransporter. Proc. Natl. Acad. Sci. June 1994, Vol. 91, pages 5607-5611. | 1-2, 7-9, 14-16 |
| A | WILSON, C. et al. Properties of a Unique Form of the Murine Amphotropic Leukemia Virus Receptor Expressed on Hamster Cells. Journal of Virology. December 1994, Vol. 68, No. 12, pages 7697-7703. | 1-4, 9-11 |
| A | US 5,288,678 A (SHU et al.) 22 February 1994. | 21-22 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02875

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/02875

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, JAPIO, BIOSIS, SCISEARCH, WPIDS, CAPLUS, EMBASE
sodium phosphate cotransport?, lithium sodium cotransport?, hpit, hpip1, hpit 2, hbnpi, lithium therapy, manic depress?, mental illness, retrovirus receptor#, nucleotide, dna, rna, cdna, mrna, blood, erythrocytes, flux analysis,lithium, cell, membrane, spectroscopy, absorption, emission, lithium analysis, countertransport.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group 1, claim(s)1-15, drawn to DNA encoding lithium-sodium countertransporter and retrovirus receptor useful as lithium-sodium countertransporter.

Group 2, claim(s) 16-20, drawn to method of evaluating sensitivity to lithium therapy in manic depressive patients comprising using hybridization with primers specific for sequences coding for lithium-sodium countertransporter.

Group 3, claim(s) 21-22, drawn to method of evaluating sensitivity to lithium therapy in manic depressive patients using flux analysis.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

1. hPiT-1 (SEQ ID NO:1 and 2)
2. hPiT-2 (SEQ ID NO:3 and 4)
3. hBNPI (SEQ ID NO:5 and 6)

The claims are deemed to correspond to the species listed above in the following manner:

1. Claims 3, 4, 10, 11 correspond to species hPiT-1.
2. Claims 5, 6, 12, 13 correspond to species hPiT-2.
3. Claims 7, 8, 14, 15 correspond to species hBNPI.

The following claims are generic: 1, 2 and 9.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:

Although, the DNA of SEQ ID NO:2, 4, 6 contain sequences that encode functionally related lithium-sodium countertransporter of corresponding SEQ ID NO:1, 3, 5, they are distinctly different proteins as can be seen by their vastly different amino acid sequences.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group I is the lithium-sodium countertransporter. These claims are considered by the ISA/US to constitute the main invention, and none of the related groups II and III correspond to the main invention.

The methods of Groups II and III do not share the same or corresponding special feature with Group I, because they do nor require the DNA or protein sequences of Group 1 to form a separate inventive concept.

Since no special technical feature of any group other than the main invention is shared by any of the other inventions, unity of invention is lacking.